

Description**FIELD OF THE INVENTION**

5 [0001] The present invention is based on the finding that antibodies directed against specific epitopes of the E1 and E2 protein of HCV can be used to detect viral antigens in host tissues.

BACKGROUND OF THE INVENTION

10 [0002] Hepatitis C virus (HCV) infection is a major health problem in both developed and developing countries. It is estimated that about 1 to 5 % of the world population is affected by the virus. HCV infection appears to be the most important cause of transfusion-associated hepatitis and frequently progresses to chronic liver damage. Moreover, there is evidence implicating HCV in induction of hepatocellular carcinoma. Consequently, the demand for reliable diagnostic methods and effective therapeutic agents is high. Also sensitive and specific screening methods of HCV-contaminated 15 blood-products and improved methods to culture HCV are needed.

[0003] HCV is a positive stranded RNA virus of approximately 9,400 bases which encode at least three structural and six non-structural proteins. Based on sequence homology, the structural proteins have been functionally assigned as one single core protein and two envelope proteins: E1 and E2. The E1 protein consists of 192 amino acids and contains 5 to 6, depending on the HCV genotype, N-glycosylation sites whereas the E2 protein consists of 363 to 370, depending 20 on the HCV genotype, amino acids containing 9-11 N-glycosylation sites (for review see Major and Feinstone, 1997, and, Maertens and Stuyver, 1997). The E1 protein contains various variable domains (Maertens and Stuyver, 1997) while the E2 protein contains a two hypervariable domains, of which the major domain is located at the N-terminus of the protein. The latter envelope proteins have been produced by recombinant techniques in *Escherichia coli*, insect cells, yeast cells and mammalian cells. The usage of an expression system in higher eukaryotes and especially in mammalian cell culture leads to envelope proteins of superior quality, i.e. they are effectively recognized by antibodies in 25 patient samples as described in PCT/EP 95/03031 to Maertens et al.

[0004] Currently, the detection of HCV in cells or tissues relies mainly on the demonstration of viral RNA. However, RNA detection in cells or tissues is a cumbersome technique which either involves the extraction of RNA followed by reverse transcription and nested PCR or includes *in situ* RT-PCR and hybridization. Moreover, these techniques are 30 prone to false positive reactivity. Consequently, viral RNA detection is performed on serum samples only. Reliable methods for the detection of viral protein antigens in serum and tissue samples, on the other hand, are still lacking.

[0005] The replication sites of HCV have not yet been fully elucidated. It is generally accepted that the virus replicates in hepatocytes, but replication in other tissues, such as lymphoid tissues, is still highly debated. A reliable method for the detection of viral proteins or the virus itself may solve this issue.

[0006] The detection of viral proteins has been hampered by the lack of antibodies which specifically bind to viral proteins and which are able to recognize the native antigens as expressed in host cells. As a consequence, only few reports relate to demonstrating the presence of viral proteins in host cells (for review see Guido and Thung, 1996). For the latter purpose, host-derived antibodies have been used in many studies. However, the latter preparations cannot be 40 easily reproduced and they may be contaminated by autoimmune antibodies as well as by antibodies against other known or even unknown agents. It is known that antibodies produced in animals upon immunization with recombinant antigens will yield antibodies with the desired specificity. In order to have reproducible quality, however, monoclonal antibodies are preferred. As the envelope proteins of HCV need to be produced by mammalian expression to yield good quality antigens, only few monoclonal antibodies have been described which could be used to detect HCV antigen in tissue specimens of patients. These antibodies were directed against the N-terminal region of E1, amino acids (aa) 192-228, (Hiramatsu et al., 1992, Kaito et al, 1994) or the C-terminal domain of E2, aa 451-715 (Saneonno et al., 45 1997(a, b)). However, from these publications and from the review of Guido and Thung, it is clear that there is still an existing need for well characterized antibodies allowing efficient and routine *in situ* detection of HCV.

[0007] Taken together, the identification of new specific epitopes on the envelope proteins which are accessible for antibodies and which allow antigen detection is needed. However, these envelope proteins are highly variable so that 50 antibodies with a high cross-reactivity towards the different genotypes of HCV are needed. The identification of such epitopes and the search for antibodies with high cross-reactivity towards the sequence variation of HCV is, however, a challenging undertaking.

[0008] The present application relates to specific monoclonal antibodies, directed against particular epitopes in the envelope proteins of HCV, which are able to detect HCV antigen in tissue specimens of patients. In total two such 55 epitopes, and corresponding antibodies, were found: one in the C-terminal region (aa 227-383) of the envelope protein E1 and one in the N-terminal hypervariable region (HVR) of E2 (aa 384-450). Although the latter region, and more specifically the region 385-415, is considered to be hypervariable, we characterized, to our surprise, an antibody which reacts with various known sequences of the HVR of E2.

AIMS OF THE INVENTION

[0009] It is clear from the literature that there is an urgent need to develop reliable diagnostic methods, reliable vaccines and effective therapeutic agents for HCV. Also sensitive and specific screening methods of HCV-contaminated

5 blood-products and improved methods to culture HCV are needed. New antibodies able to detect the virus in animal- or *in vitro* models, or in its natural host, may help in designing efficient diagnostic tools and therapeutic agents. In this regard, the present invention is based on the surprising finding of monoclonal antibodies directed against either E1 or E2-HVR which can be used for the detection of HCV antigens in various tissues or cells. These tissues include the liver 10 but also cells derived from blood samples. Therefore, the present invention aims at providing an antibody specifically binding to HCV envelope protein region aa 227-450, which covers the main part (C-terminal) of the E1 protein (the complete E1 protein corresponds to aa 192-383; see Major and Feinstone, 1997, and, Maertens and Stuyver, 1997) and the N-terminal region of the E2 protein (the complete E2 protein corresponds to aa 384-747; see Major and Feinstone, 1997, and, Maertens and Stuyver, 1997) which allows the *in situ* detection of HCV protein antigens.

[0010] More specifically, the present invention aims at providing an antibody as defined above which specifically binds 15 to at least one of the following epitopes:

- aa 307-326 of HCV E1 protein (SEQ ID 30)
- aa 398-415 of HCV E2 protein (SEQ ID 31).

[0011] Moreover, the present invention aims at providing an antibody as defined above which is a monoclonal antibody. In this regard, the present invention aims at providing a monoclonal antibody secreted by the hybridoma line with 20 ECACC deposit having provisional accession number 98031215 or 98031214.

[0012] It should be clear that the present invention also aims at providing any functionally equivalent variant or fragment of any antibody as defined above, as well as mutant forms thereof or molecules exhibiting similar functional binding reactivities with SEQ ID 30 and 31, such as sequences obtained from phage- or other libraries.

[0013] In addition, the present invention aims at providing a hybridoma cell line secreting a monoclonal antibody which 25 specifically binds to HCV E1 protein (aa 227-383) or HCV E2 N-terminal hypervariable region (aa 384-450) and which allows the *in situ* detection of HCV protein antigens. More specifically, the present invention aims at providing the hybridoma cell line corresponding to the ECACC deposit having provisional accession number 98031215 or 98031214.

[0014] Furthermore, the present invention also aims at providing a method for the *in situ* detection of HCV protein 30 antigens comprising:

- contacting a test sample which may contain HCV protein antigens with an antibody as defined above or with a functionally equivalent variant or fragment of said antibody, to form an antibody-antigen complex, and
- determining said antigen-antibody complex with an appropriate marker.

[0015] More specifically, the present invention aims at providing a method as defined above wherein said test sample 35 comprises human cells, such as peripheral blood cells, or tissues, such as liver tissue.

- [0016] Finally, the present invention aims at providing an assay kit for the *in situ* detection of HCV protein antigens comprising:

[0017] All the aims of the present invention are considered to have been met by the embodiments as set out below.

BRIEF DESCRIPTION OF TABLES AND DRAWINGS

[0018]

50 Table 1 provides the peptide sequences of all peptides mentioned in this application.

Table 2 shows the cross-reactivity of IGH 222 towards various sequences of the hyper variable domain in E2. All peptides were biotinylated, bound to streptavidin coated microliterplates and allowed to react with IGH 222.

55 Figure 1 shows staining of E2 antigen, revealed by the monoclonal antibody IGH 222, on a liver biopsy of an HCV patient. Immunohistochemistry was performed on 4 µm tick cryostat sections of fresh frozen materials using a three step indirect immuno-peroxidase procedure. Sections were incubated overnight at 4°C with monoclonal antibody IGH 222 (purified IgG₁; 10 ng/µl). The secondary and tertiary antibodies consisted of peroxidase-conjugated rabbit

anti-mouse and peroxidase-conjugated swine anti-rabbit IgG, respectively (both obtained from Dakopatts, Copenhagen, Denmark; working dilution 1/50 and 1/100, respectively). Each incubation was performed 30 minutes at room temperature and followed by a wash in three changes of phosphate buffered saline, pH 7.2. The reaction product was developed by incubation for 15 minutes in 100 mM acetate buffer (pH 5.2), containing 0.05% 3-amino-9-ethyl-carbazole and 0.01% H₂O₂, resulting in bright red staining of immuno-reactive sites. Controls (not shown) consisted of irrelevant monoclonal antibodies of similar isotype as the primary antibody, or of chromogen alone: these controls were consistently negative. The photograph shows a 25X magnification. The darkest staining reveals the presence of HCV antigen in hepatocytes only (see arrows).

Figure 2 shows staining of E1 antigen, revealed by the monoclonal antibody IGH 207, on a liver biopsy of an HCV patient. The procedure followed is identical to the one described in figure 1, except for the concentration of the monoclonal antibody which was 30 ng/μl. The photograph shows a 10X magnification on which the staining of the cells in the lymphocyte infiltrates is dominant. The darkest staining reveals the presence of HCV antigen in hepatocytes (see arrow) and in infiltrating lymphocytes (see double arrow).

Figure 3 shows staining, revealed by the monoclonal antibody IGH 207, of intracellular E1 antigen in peripheral blood mononuclear cells. Peripheral white blood cells (0.5x10⁶) were suspended in 200 μl PBS-0.1% saponin, 2 μg of IGH 207 was added and allowed to react for 25 minutes at 4°C. The cells are washed three times with 8 ml PBS-0.1% saponin and three times with PBS-0.2% NaN₃. Finally cells are resuspended in 250 μl of PBS-0.2% NaN₃ and analyzed by flow cytometry. After gating on the mononuclear cell fraction (right column), the fluorescence was plotted (left column). Samples 1-5 are derived from HCV chronic carriers while sample 6 is derived from a healthy blood carrier. The left column shows two examples of the gating on the mononuclear cell fraction (samples 2 and 6), while the right column shows the fluorescence found in these mononuclear cells. While the control sample shows no staining at all with this monoclonal antibody, there is a marked positivity in all HCV patients, except for patient 4 for whom a weaker signal was obtained.

Figure 4 shows staining, revealed by the monoclonal antibody IGH201, of intracellular E1 antigen in peripheral blood mononuclear cells. The technique was similar as described for Figure 3. Samples 7-11 are derived from HCV chronic carriers while sample 12 is derived from a healthy blood carrier. The left column shows two examples of the gating on the mononuclear cell fraction (samples 7 and 12), while the right column shows the fluorescence found in these mononuclear cells. Although the control sample reveals a higher background staining, the reaction in the patient samples can be easily discriminated based on the two populations which can be seen: a population with a staining similar as in the control and a second population with high intensity staining, not seen in the control.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The invention described herein draws on previously published work and pending patent applications. By way of example, such work consists of scientific papers, patents or pending patent applications. All these publications and applications, cited previously or below are hereby incorporated by reference.

[0020] It is clear that the detection of viral proteins has been hampered by the lack of antibodies which specifically bind to viral proteins and which are able to recognize the native antigens as expressed in host cells. The present invention is based on the finding of two new epitopes on the HCV envelope proteins which allow routine detection of native HCV envelope antigens, by means of antibodies directed against these epitopes, in tissue or cells derived from the host. Thus, the present invention relates to an antibody specifically binding to the C-terminal region of HCV E1 protein (aa 227-383) or HCV E2 N-terminal hypervariable region (aa 384-450) which allows the *in situ* detection of HCV protein antigens. The terms "antibody specifically binding to the C-terminal region of HCV E1 protein (aa 227-383) or HCV E2 N-terminal hypervariable region (aa 384-450)" refer to any polyclonal or monoclonal antibody binding to an hepatitis C viral particle or any molecule derived from said viral particle, more particularly to the C-terminal region of the E1 protein and the N-terminal region of the E2 protein. The "envelope region" of the latter viruses, and thus the "HCV E1 C-terminal region (aa 227-383) or HCV E2 N-terminal hypervariable (aa 384-450) regions" are well-known regions by a person skilled in the art (Wengler, 1991; Major and Feinstone, 1997; Maertens and Stuyver, 1997). The term "bind" indicates that the antibodies of the present invention are physically connected to the HCV proteins. The term "monoclonal antibody" used herein refers to an antibody composition having a homogeneous antibody population. The term is not limiting regarding the species or source of the antibody, nor is it intended to be limited by the manner in which it is made. In addition, the term "antibody" also refers to humanized antibodies in which at least a portion of the framework regions of an immunoglobulin are derived from human immunoglobulin sequences and single chain antibodies as described in U.S. patent N° 4,945,778 and to fragments of antibodies such as F_{ab}, F_{(ab)2}, F_v and other fragments which retain the antigen binding function and specificity of the parent antibody. Also included in the term "antibody" are diabodies, tri-

bodies and tetravalent antibodies, as described in EP application N° 97670092.0 to Lorré et al., which retain the antigen binding function and specificity of the parent antibody.

[0021] The terms "*in situ* detection of HCV protein antigens" refer to the detection of any HCV protein antigen, in particular to the detection of the C-terminal region of HCV E1 (aa 227-383) protein or the HCV E2 N-terminal hypervariable (aa 384-450) region, in their natural or original position. In other words, the latter terms refer to the visualisation of the presence of the C-terminal region of HCV E1 (aa 227-383) protein or the HCV E2 N-terminal hypervariable (aa 384-450) region in, or on, their natural host cell or tissue by means of the antibodies of the present invention. The natural host cell or tissue can be any host cell or tissue derived from any host species. In particular, the natural host cell refers to peripheral blood cells and the natural tissue refers to liver tissue (see also Examples section of the present application). The natural host refers, in particular, to humans but may also refer to non-human primates or other mammals.

[0022] More specifically, the present invention relates to antibodies which specifically bind to at least one of the fol-

following epitopes: aa 307-326 of HCV E1 protein and aa 395-415 of HCV E2 protein. The latter antibodies are secreted by hybridomas deposited at the European Collection of Cell Cultures (ECACC), Centre for Applied Microbiology & Research, Salisbury, Wiltshire SP4 0JG, U.K. (Tel: +44 1980 612512; fax: +44 1980 611315) on March 12, 1998, which

aa310-322, aa311-323, aa312-324, aa313-325, aa314-326, aa307-320, aa308-321, aa309-322, aa310-323, aa311-324, aa312-325, aa313-326, aa307-321, aa308-322, aa309-323, aa310-324, aa311-325, aa312-326, aa307-322, aa308-323, aa309-324, aa310-325, aa311-326, aa312-327, aa307-323, aa308-324, aa309-325, aa310-326, aa307-324, aa308-325, aa309-326, aa310-327, aa307-325, aa308-326, and,

aa400-406, aa401-407, aa402-408, aa403-409, aa404-410, aa405-411, aa406-412, aa407-413, aa408-414, aa409-415, aa395-403, aa396-402, aa397-404, aa398-405, aa399-406

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aa487-488, aa488-489, aa489-490, aa490-491, aa491-492, aa492-493, aa493-494, aa494-495, aa495-496, aa496-497, aa497-498, aa498-499, aa499-500, aa500-501, aa501-502, aa502-503, aa503-504, aa504-505, aa505-506, aa506-507, aa507-508, aa508-509, aa509-510, aa510-511, aa511-512, aa512-513, aa513-514, aa514-515, aa515-516, aa516-517, aa517-518, aa518-519, aa519-520, aa520-521, aa521-522, aa522-523, aa523-524, aa524-525, aa525-526, aa526-527, aa527-528, aa528-529, aa529-530, aa530-531, aa531-532, aa532-533, aa533-534, aa534-535, aa535-536, aa536-537, aa537-538, aa538-539, aa539-540, aa540-541, aa541-542, aa542-543, aa543-544, aa544-545, aa545-546, aa546-547, aa547-548, aa548-549, aa549-550, aa550-551, aa551-552, aa552-553, aa553-554, aa554-555, aa555-556, aa556-557, aa557-558, aa558-559, aa559-560, aa560-561, aa561-562, aa562-563, aa563-564, aa564-565, aa565-566, aa566-567, aa567-568, aa568-569, aa569-570, aa570-571, aa571-572, aa572-573, aa573-574, aa574-575, aa575-576, aa576-577, aa577-578, 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aa942-943, aa943-944, aa944-945, aa945-946, aa946-947, aa947-948, aa948-949, aa949-950, aa950-951, aa951-952, aa952-953, aa953-954, aa954-955, aa955-956, aa956-957, aa957-958, aa958-959, aa959-960, aa960-961, aa961-962, aa962-963, aa963-964, aa964-965, aa965-966, aa966-967, aa967-968, aa968-969, aa969-970, aa970-971, aa971-972, aa972-973, aa973-974, aa974-975, aa975-976, aa976-977, aa977-978, aa978-979, aa979-980, aa980-981, aa981-982, aa982-983, aa983-984, aa984-985, aa985-986, aa986-987, aa987-988, aa988-989, aa989-990, aa990-991, aa991-992, aa992-993, aa993-994, aa994-995, aa995-996, aa996-997, aa997-998, aa998-999, aa999-1000, aa1000-1001, aa1001-1002, aa1002-1003, aa1003-1004, aa1004-1005, aa1005-1006, aa1006-1007, aa1007-1008, aa1008-1009, aa1009-1010, aa1010-1011, aa1011-1012, aa1012-1013, aa1013-1014, aa1014-1015, aa1015-1016, aa1016-1017, aa1017-1018, aa1018-1019, aa1019-1020, aa1020-1021, aa1021-1022, aa1022-1023, aa1023-1024, aa1024-1025, aa1025-1026, aa1026-1027, 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aa397-408, aa398-410, aa399-411, aa400-412, aa401-413, aa402-414, aa403-414, aa404-415, aa395-407, aa396-408, aa397-409, aa398-410, aa399-411, aa400-412, aa401-413, aa402-414, aa403-415, aa395-408, aa396-409, aa397-410

414, aa387-415, aa395-414 and 396-415, 397-398. The necessary material is given in the following table.

[0023] The present invention also relates to functionally equivalent variants or fragments of the above indicated antibodies. The terms "functionally equivalent variants or isomeric" refer to any variant or isomeric form of an antibody which retains the same biological activity as the original antibody.

antibodies which retain the antigen binding function and specificity of the parent antibody. More specifically, the latter

terms refer to humanized antibodies and single chain antibodies as defined above and to fragments of antibodies such as F_{ab} , F_{ab2} , F_v and the like. Also included are diabodies, triabodies and tetravalent antibodies, as described above, as well as mutant forms thereof or molecules exhibiting similar functional binding reactivities with SEQ ID 30 and 31, such as sequences obtained from phage - or other libraries as described by Ladher, 1995; MacLennan, 1995 and Can-

non et al., 1996. Indeed, any peptide described by the latter authors, constrained or not, which allows the *in situ* detection of HCV envelope proteins is part of the present invention.

[0024] The present invention also relates to hybridoma cell lines secreting antibodies as defined above. In this regard, it should be clear that the hybridoma technology for obtaining mAb's is well known to a person skilled in the art. It should also be clear that mapping the epitopes to which the mAb's specifically bind can be performed by any method known in the art such as the ones described in PCT/EP 97/07268 to Depla et al.

[0025] The present invention also relates to a method for the *in situ* detection of HCV protein antigens comprising:

- contacting a test sample which may contain HCV protein antigens with an antibody as defined above or with a functionally equivalent variant or fragment of said antibody, to form an antibody-antigen complex, and
- determining said antigen-antibody complex with an appropriate marker.

[0026] The term "test sample" can be any sample obtained from an organism, such as serum, plasma, saliva, mucus, sections or biopsies taken from any tissue or organ such as liver biopsies, skin biopsies and the like. The term biopsy specifically refers to a sample comprising human cells, such as peripheral blood cells, or tissues, such as liver tissue.

[0027] The terms "determining said antigen-antibody complex with an appropriate marker" refer to any method known in the art which detects, or visualises, the above-indicated antigen-antibody complexes, such as fluorescence flow cytometry, binding-, ELISA- and RIA-type assays or competition assays (see Examples section, Hertogs et al., 1993, and, WO 93/04084 to Mehta et al.). Similarly, the term "appropriate marker" refers to any marker known in the art such as the ones described in WO 93/04084 to Mehta et al id Coligan et al, 1992 which visualises the above-indicated antigen-antibody complexes. In this regard, the present invention also relates to an assay kit for the *in situ* detection of HCV protein antigens comprising: an antibody as defined above, or, a functionally equivalent variant or fragment of said antibody, and appropriate markers which allow to determine the complexes formed between HCV protein antigens in a test sample with said antibody or a functionally equivalent variant or fragment of said antibody.

[0028] The present invention will now be illustrated by reference to the following examples which set forth particularly advantageous embodiments. However, it should be noted that these embodiments are illustrative and can not be construed as to restrict the invention in any way.

EXAMPLES

Example 1: Generation of monoclonal antibodies against E1 and E2.

[0029] Mice were immunized with truncated versions of E1 (aa 192-326) and E2 (aa 364-678) expressed by recombinant vaccinia virus, as described in PCT/EP 95/09031 to Maartens et al. After immunization splenocytes of the mice were fused with a myeloma cell line. Resulting hybridomas secreting specific antibodies for E1 or E2 were selected by means of ELISA.

Example 2: Selection of monoclonal antibodies.

[0030] A large series (25 in total) of monoclonals directed against E1 or E2 was evaluated for staining of native HCV antigen in liver biopsies of HCV patients and controls. Only two monoclonal antibodies revealed a clear and specific staining. All other monoclonal antibodies either gave no staining at all or showed non-specific staining. Remarkably, both antibodies revealed different antigen staining patterns. Monoclonal IGH 222, directed against E2, clearly stained hepatocytes (Figure 1) while IGH 207, directed against E1, stained hepatocytes to a weaker degree, while marked staining of lymphocyte infiltrates in the liver was seen (Figure 2). This staining pattern was confirmed on a series of biopsies of five different patients.

Example 3: Identification of monoclonal antibodies allowing detection of viral envelope antigen in peripheral blood cells.

[0031] The finding that lymphocyte infiltrates in the liver can be stained for HCV envelope antigens prompted us to look also at peripheral blood mononuclear cells. In order to allow intracellular staining, peripheral blood cells were permeabilized with saponin, allowed thereafter to react with the monoclonal antibodies, and finally reactivity was checked on a fluorescent cell sorter using secondary FITC-labelled antibodies. Two monoclonals were found showing specific staining in several HCV patients. IGH 207, directed against E1 and which stained already the lymphocyte infiltrates in the liver, showed a high specificity. With this monoclonal, almost no background staining was detected and 4 out of 5 patients clearly stained positive (Figure 3). The second monoclonal, IGH 201 (SEQ ID 29; ECACC provisional accession number: 98031216) which is also directed against E1, yields a higher background but intracellular E1 was detected

in 5 out of 5 patients as can be deduced from the histograms presented in Figure 4, which show a clear subpopulation of cells with a higher degree of fluorescence as compared to the control sample.

Example 4: Mapping of the reactive monoclonal antibodies against E1 or E2

[0032] The monoclonal antibodies IGH 201, 207 and 222 were mapped to their respective epitopes using peptides scanning the E1 and E2 protein against which the antibodies were raised. These peptides were biotinylated, bound to streptavidin sensitized microtiterplates and allowed to react with the monoclonal antibodies. As a positive control recombinant envelope proteins were checked.

10 For each monoclonal antibody reactivity could be assigned to a specific epitope region defined by two overlapping peptides (for details on sequences see Table I).

peptides	aa region	reactive monoclonals
V1V2	192-226	IGH 201
V2V3	212-244	IGH 201
V3V4	230-263	
HR	261-290	
V3C4	288-327	IGH 207
C4V6	307-340	IGH 207
recombinant	192-326	IGH 201 and 207
HVR I	384-415	IGH 222
HVR1/C1a	395-428	IGH 222
C1a	418-447	
C1b	430-467	
HVRII	460-487	
C2a	480-513	
C2b	500-530	
V3-C3	523-556	
V4	561-590	
C4a	578-627	
C4b	621-648	
C4c	641-673	
recombinant	384-673	IGH 222

50 The epitope for IGH 201 can be defined as the region 212-226 (SEQ ID 29), for IGH 207 this is 307-326 (SEQ ID 30) and for IGH 222 this is 385-415 (SEQ ID 31). The amino acid region of IGH 201 is a rather variable region of the E1 protein of HCV and has already been previously reported in relation to *in situ* detection of HCV (Hiramatsu et al., 1992, Keito et al., 1994). However, from our studies it is clear that antibodies directed against this epitope are less suitable for *in situ* detection of HCV as the liver biopsy staining with this antibody was-negative and the staining on peripheral blood lymphocytes showed background. In contrast, IGH 207, which recognizes a completely conserved region of E1 (Mae-rtens and Stuyver, 1997) and IGH 222, which recognizes a region of E2 which is part of the N-terminal hypervariable domain of E2, proved to be very suitable for efficient *in situ* detection of HCV.

Example 5: Determination of cross-reactivity on variable epitopes

[0033] Using an extended series of peptides derived from various sequences of the N-terminal hypervariable epitope

of E2, the IGH 222 was further characterized. Table 2 shows a summary of these experiments. From this table it can be concluded that this monoclonal reacts with several sequences, but fails to react with some others. Knowing this epitope is sufficient for the man skilled in the art to raise additional antibodies against this epitope with a better reactivity towards other sequences which are currently not recognized by IGH 222. Such sequences are by way of example the peptides with # 490, 940, 884, 484 and 494 but other sequences in the region between aa 395-415 may be found against which IGH 222 may fail to react.

[0034] From these examples it is clear that the epitopes recognized by the monoclonal antibodies IGH 207 and 222 are readily accessible for binding antibodies and allow detection of the antigen in liver biopsies and peripheral blood cells. Although the properties of the monoclonal antibodies IGH 207 and 222 are rather unique (other monoclonals directed against the same epitopes resulted in high background and absence of specific staining) it should be feasible for the man skilled in the art to produce large series of antibodies, either polyclonal or monoclonal of nature, in various species. The production of such large series of antibodies will allow to identify some of them as having similar properties as IGH 207 and 222 i.e. to be able to reveal the presence of HCV envelope protein in tissue samples of the host.

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Table 1

E1 peptides	Genotype	name	#	aa	Seq ID
YEVRNVSGIYHVTNDCSNSSIVYEADMIMHTPGC	1b	V1V2	888	192-226	1
IVYEADMIMHTPGCVPVCVRENNSSRCWV	1b	V2V3	1036	212-244	2
VRENNSSRCWVALPTLAARNASVPTTIRRHVD	1b	V3V4	1022	230-263	3
HVDLLVGAAAFCSAMYVGDLCCGSVFLVSQL	1b	HR	1150	261-290	4
SQLFTISPRPRHETVQOCNCISIYPGHITCH- RMAWDMMMNWS	1b	V5C4	1176	286-327	5
SIYPGHITCHGHRMAMWDMMMNWSPTTALVVSQLLRI	1b	C4V6	1039	307-340	6
E2 peptides					
HTRVSGGAAASNTRQLVSLFSPG6AQKIQLVN	1b	HVR I	1139	384-415	7
NTRGLVSLFSPGSAQKIQLVNTNG5WHINRTALN	1b	HVR I C1a	1173	395-428	8
LVNTNG5WHINRTALNNDLSLOTQFFAALFYKHKF	1b	C1a	1143	413-447	9
NDSLQTGFFAALFYKHKFNFNSQOPERLAS- CRSIDKFAQ	1b	C1b	1148	430-467	10
RSIDKFAQGWGQPLTYTEPNSSDQRPYCW	1b	HVR II	1020	460-487	11
SDQRPYCWHYAPRPGIVPASQVCGPVYCFTPSP	1b	C2a	1147	480-513	12
SQVQGPVYCFTPSPVVVGTTDRFGVPTYNWG	1b	C2b	1143	530-560	13
GVPTYNWGANDSDVLILNNNTRPPRGNWF- GCTWMNGTGFTKTCQG	1b	V3-C3	1178	523-566	14
TKTCGGPPCNIQGAGNNLTCTDQPRKHP	1b	V4	1142	561-590	15
TDCFRKHPEATYARQGSGPWLTPROM- VHYPYRLWHYPCTVNFIF	1b	C4a	18	583-627	16
TVNFTIFKVRMYVQGVHHRFEACNWTR	1b	C4b	1141	621-648	17
EAACNWTRGERCDLEORDRSELSPLLLSTTEWQ	1b	C4c	1140	641-673	18
KTITNRLVSMFASGPKQNVHLINT	-	HVR I	485	394-416	19
HTTSTLASLFLSPGASQRIQLVNT	-	HVR I	492	395-416	20
HVTCTLTSLFRPGASQKIQLVNT	-	HVR I	483	394-416	21
AHNARTLTGMSLQARQKIQLINT	-	HVR I	520	394-416	22
SDTRGLVSLFSPGSAQKIQLVNT	-	HVR I	886	394-416	23
SSTQSLVSWLSQGPQKIQLVNT	-	HVR I	494	394-416	24
HTMTGIVRFFAPGPKQNVHLINT	-	HVR I	484	394-416	25
RAMSGGLVSLFTPQAKQNIQLINT	-	HVR I	884	394-416	26
HYTGTTLTSLFRPGASQKIQLVNT	-	HVR I	940	394-416	27

Table 2

sequence ID	#	aa region	Recognition by IGH 222	Seq
HTRVSGGAAASNTRQLVSLFSPGSAQKIQLVN	1139	394-415	+	7
KTTRNLVSMFASGPKQNVHLINT	485	394-416	+	13
HTTSTLASLFSFGASGRIQLVNT	492	395-416	+	20
AHNARTITGMFSLGARQKIQLVNT	520	394-416	+	22
SDTRQLVSLFSPGSAQKIQLVNT	886	394-416	+	23
SSTQSLVSNLSQLPSQKIQLVNT	494	394-416	-	24
HTMTGIVRFFFAPGPKQNVHLINT	484	394-416	-	25
RAMSQLVSLFTPQAKQNIQLINT	884	394-416	-	26
HVTGTLTSLFRPGASQKIQLVNT	940	394-416	-	27
RTTQQQLVSLFSRQAKQDIQLINT	490	394-416	-	28

Non-conservative mutations in the peptides not reacting with IGH 222 are shown in bold

SEQUENCE LISTING

5. (1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: INNOGENETICS NV
 (B) STREET: INDUSTRIEPARK ZWIJNAARDE NR 7, BOX 4
 (C) CITY: GHENT
 (D) COUNTRY: BELGIUM
 (F) POSTAL CODE (ZIP): B9052
 (G) TELEPHONE: 32 9 241 87 11
 (H) TELEFAX: 32 9 241 87 93

10. (ii) TITLE OF INVENTION: Epitopes in viral envelope proteins and
 specific antibodies directed against these epitopes: use
 for detection of HCV viral antigen in host tissue.

15. (iii) NUMBER OF SEQUENCES: 31

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

25. (2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

40. (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Y₁ Tyr Glu Val Arg Asn Val Ser G₅ly Ile Tyr His Val₁₀ Thr Asn Asp Cys₁₅

Ser Asn Ser Ser Ile Val Tyr Glu Ala Ala Asp Met Ile Met His Thr₂₀
₂₅ 30

Pro Gly Cys
₃₅

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 amino acids

2
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10
 (iii) MOLECULE TYPE: peptide

(iv) HYPOTHETICAL: NO

15
 (v) ANTI-SENSE: NO

18
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Ile Val Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys Val
 1 5 10 15
 Pro Cys Val Arg Glu Asn Asn Ser Ser Arg Cys Trp Val
 20 25

20
 (2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 34 amino acids
 (B) TYPE: amino acid
 25
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30
 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

35
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Val Arg Glu Asn Asn Ser Ser Arg Cys Trp Val Ala Ile Thr Pro Thr
 1 5 10 15
 Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Thr Ile Arg Arg His
 20 25 30
 Val Asp

40
 (2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 amino acids
 (B) TYPE: amino acid
 45
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5 (iii) HYPOTHETICAL: NO

10 (iv) ANTI-SENSE: NO

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

16 His Val Asp Leu Leu Val Gly Ala Ala Ala Phe Cys Ser Ala Met Tyr
 1 5 10 15

20 Val Gly Asp Leu Cys Gly Ser Val Phe Leu Val Ser Glu Leu
 25 30

25 (2) INFORMATION FOR SEQ ID NO: 5:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: peptide

40 (iii) HYPOTHETICAL: NO

45 (iv) ANTI-SENSE: NO

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

55 Ser Glu Leu Phe Thr Ile Ser Pro Arg Arg His Glu Thr Val Glu Asp
 1 5 10 15

60 Cys Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg Met Ala
 20 25 30

65 Trp Asp Met Met Met Asn Trp Ser
 35 40

70 (2) INFORMATION FOR SEQ ID NO: 6:

75 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

80 (ii) MOLECULE TYPE: peptide

85 (iii) HYPOTHETICAL: NO

90 (iv) ANTI-SENSE: NO

8 (1) SEQUENCE DESCRIPTION: SEQ ID NO: 6;

9 Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg Met Ala Trp Asp Met
 1 5 10 15

10 Met Met Asn Trp Ser Pro Thr Thr Ala Leu Val Val Ser Gln Leu Leu
 20 25 30

11 Arg Ile

12 (2) INFORMATION FOR SEQ ID NO: 7;

13 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

14 (ii) MOLECULE TYPE: peptide

15 (iii) HYPOTHETICAL: NO

20 (iv) SEQUENCE DESCRIPTION: SEQ ID NO: 7;

21 His Thr Arg Val Ser Gly Gly Ala Ala Ala Ser Asn Thr Arg Gly Ieu
 1 5 10 15~

22 Val Ser Leu Phe Ser Pro Gly Ser Ala Gln Lys Ile Gln Leu Val Asn
 20 25 30

25 (2) INFORMATION FOR SEQ ID NO: 8;

26 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

31 (iii) HYPOTHETICAL: NO

35 (iv) ANTI-SENSE: NO

40 (v) SEQUENCE DESCRIPTION: SEQ ID NO: 8;

41 Asn Thr Arg Gly Ieu Val Ser Leu Phe Ser Pro Gly Ser Ala Gln Lys
 1 5 10 15

42 Ile Gln Leu Val Asn Thr Asn Gly Ser Trp His Ile Asn Arg Thr Ala
 20 25 30

Leu Asn

5 (2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Leu	Val	Asn	Thr	Asn	Gly	Ser	Trp	His	Ile	Asn	Arg	Thr	Ala	Leu	Asn
1									5						10

16

16

Cys	Asn	Asp	Ser	Leu	Gln	Thr	Gly	Phe	Phe	Ala	Ala	Leu	Phe	Tyr	Lys
									20					25	30

His	Lys	Phe
-----	-----	-----

35

30 (3) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Asn	Asp	Ser	Leu	Gln	Thr	Gly	Phe	Phe	Ala	Ala	Leu	Phe	Tyr	Lys	His
1									5					10	15

10

15

Lys	Phe	Asn	Ser	Ser	Gly	Cys	Pro	Glu	Arg	Leu	Ala	Ser	Cys	Arg	Ser
									20					25	30

Ile	Asp	Lys	Phe	Ala	Gln
-----	-----	-----	-----	-----	-----

35

(2) INFORMATION FOR SEQ ID NO: 11:

5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 28 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

16

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

20 Arg Ser Ile Asp Lys Phe Ala Gln Gly Trp Gly Pro Leu Thr Tyr Thr
 1 5 10 15
 Glu Pro Asn Ser Ser Asp Gln Arg Pro Tyr Cys Trp
 25 29 25

(2) INFORMATION FOR SEQ ID NO: 12:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 34 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

36

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

40 Ser Asp Gln Arg Pro Tyr Cys Trp His Tyr Ala Pro Arg Pro Cys Gly
 1 5 10 15
 Ile Val Pro Ala Ser Gln Val Cys Gly Pro Val Tyr Cys Phe Thr Pro
 20 25 30
 45 Ser Pro

(2) INFORMATION FOR SEQ ID NO: 13:

50 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single

56

8 (D) TOPOLOGY: linear

9 (iii) MOLECULE TYPE: peptide

10 (iii) HYPOTHETICAL: NO

11 (iv) ANTI-SENSE: NO

12

13 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

14 Ser Gin Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser Pro Val Val
 1 5 10 15
 Val Gly Thr Thr Asp Arg Phe Gly Val Pro Thr Tyr Asn Trp Cys
 20 25 30

16 (2) INFORMATION FOR SEQ ID NO: 14:

17 (i) SEQUENCE CHARACTERISTICS:

18 (A) LENGTH: 44 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: peptide

22 (iii) HYPOTHETICAL: NO

24 (iv) ANTI-SENSE: NO

26

28 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

30 Gly Val Pro Thr Tyr Asn Trp Gly Ala Asn Asp Ser Asp Val Ile
 1 5 10 15
 Leu Asn Asn Thr Arg Pro Pro Arg Gly Asn Trp Phe Gly Cys Thr Trp
 20 25 30
 Met Asn Gly Thr Gly Phe Thr Iys Thr Cys Gly Gly
 35 40

42 (2) INFORMATION FOR SEQ ID NO: 15:

44 (i) SEQUENCE CHARACTERISTICS:

46 (A) LENGTH: 38 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

48 (ii) MOLECULE TYPE: peptide

50 (iii) HYPOTHETICAL: NO

52

(a) ANTI-SENSE: NO

18.11. SEQUENCE DESCRIPTION: SEQ ID NO: 15.

Thr Lys Thr Cys Gly Gly Pro Pro Cys Asn Ile Gly Gly Ala Gly Asn
 1 5 10 15
 Asn Thr Leu Thr Cys Pro Thr Asp Cys Phe Arg Lys His Pro
 20 25 30

(2) INFORMATION FOR SEQ ID NO: 167

1.2. SEQUENCE CHARACTERISTICS

- (A) LENGTH: 45 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(i.e.) MOLECULE TYPE: peptide

(245) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 146

Thr Asp Cys Phe Arg Lys His Pro Glu Ala Thr Tyr Ala Arg Cys Gly
 2 S 10 18

Ser Gly Pro Trp Ieu Thr Pro Arg Cys Met Val His Tyr Pro Tyr Arg
30 35 36

Lys Trp His Tyr Pro Cys Thr Val Asn Phe Thr Ile Phe
35 40 45

(2) INFORMATION FOR SEQ. ID. NO: 17:

(2) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(4.1) MOLECULES/TYPES: peptides

CLINICAL HYPOTHETICAL: NO.

(iv) ANTI-SENSE: NO

(83) SEQUENCE DESCRIPTION: SEQ ID NO: 371

1 Lys Val Asn Phe Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly Val
 5 6 10 15

5 Glu His Arg Phe Glu Ala Ala Cys Asn Trp Thr Arg
 10 15 20 25

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18.

25 Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu Asp
 1 5 10 15

Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Ser Thr Thr Glu Trp
 30 35 40 45

50 Glu.

(3) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19.

50 Lys Thr Thr Asn Arg Leu Val Ser Met Phe Ala Ser Gly Pro Lys Glu
 1 5 10 15

55 Asn Val His Leu Ile Asn Thr
 20

(2) INFORMATION FOR SEQ ID NO: 20:

8 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

18 (ii) MOLECULE TYPE: peptide

19 (iii) HYPOTHETICAL: NO

20 (iv) ANTI-SENSE: NO

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

20 His Thr Thr Ser Thr Leu Ala Ser Leu Phe Ser Pro Gly Ala Ser Gln
 1 5 10 15
 Arg Ile Gln Leu Val Asn Thr
 20

25 (2) INFORMATION FOR SEQ ID NO: 21:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

35 (iii) HYPOTHETICAL: NO

36 (iv) ANTI-SENSE: NO

30

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

40 His Val Thr Cys Thr Leu Thr Ser Leu Phe Arg Pro Gly Ala Ser Gln
 1 5 10 15
 Lys Ile Gln Leu Val Asn Thr
 20

45

(2) INFORMATION FOR SEQ ID NO: 22:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: peptide

55

8 (iii) HYPOTHETICAL: NO

9 (iv) ANTI-SENSE: NO

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

11 Ala His Asn Ala Arg Thr Leu Thr Gly Met Phe Ser Leu Gly Ala Arg
1 5 10 15
12 Gln Lys Ile Gln Leu Ile Asp Thr
13 20

14 (2) INFORMATION FOR SEQ ID NO: 23:

15 (i) SEQUENCE CHARACTERISTICS:

16 (A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

17 (ii) MOLECULE TYPE: peptide

18 (iii) HYPOTHETICAL: NO

19 (iv) ANTI-SENSE: NO

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

21 Ser Asp Thr Arg Gly Leu Val Ser Leu Phe Ser Pro Gly Ser Ala Gln
2 5 10 15
22 Lys Ile Gln Leu Val Asn Thr
23 20

24 (2) INFORMATION FOR SEQ ID NO: 24:

25 (i) SEQUENCE CHARACTERISTICS:

26 (A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

27 (ii) MOLECULE TYPE: peptide

28 (iii) HYPOTHETICAL: NO

29 (iv) ANTI-SENSE: NO

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

50 Ser Ser Thr Gln Ser Leu Val Ser Trp Leu Ser Gln Gly Pro Ser Gln
 1 5 10 15 20 25

55 Lys Ile Gln Ile Val Asn Thr
 20

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30 (iii) MOLECULE TYPE: peptide

35 (iii) HYPOTHETICAL: NO

40 (iv) ANTI-SENSE: NO

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

50 His Thr Met Thr Gly Ile Val Arg Phe Ala Pro Gly Pro Lys Gln
 1 5 10 15

55 Asn Val His Leu Ile Asn Thr
 20

55

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

60 (iii) MOLECULE TYPE: peptide

65 (iii) HYPOTHETICAL: NO

70 (iv) ANTI-SENSE: NO

75 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

80 Arg Ala Met Ser Gly Leu Val Ser Leu Phe Thr Pro Gly Ala Lys Gln
 1 5 10 15 20

85 Asn Ile Gln Leu Ile Asn Thr
 20

90 (2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(35) MOLECULE TYPE: peptide

СЕВЕРНЫЕ МАРСИАНСКИЕ ОБЪЕКТЫ

(iv) ANTI-SENSE: NO

(x4) SEQUENCE DESCRIPTION: SEQ ID NO: 27.

His Val Thr Gly Thr Leu Thr Ser Leu Phe Arg Pro Gly Ala Ser Glu
1 8 10 12

Lys Ile Gln Leu Val Asn Thr
20

(2) INFORMATION FOR SSO ID NO: 28.

(2) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

1.1.1 MOLECULE TYPE: peptide

(THIS HYPOTHETICAL) NO

(iv) ANTI-SENSE: NO

(23) SEQUENCE DESCRIPTION: SEQ ID NO: 20

Arg Thr Thr Glu Gly Leu Val Ser Leu Phe Ser Arg Gly Ala Lys Glu

Asp Ile Gln Leu Ile Ser Thr

134 INFORMATION AND COMMUNICATIONS

10.1002/1097-0258(199903)16:3<303::AID-CPD100>3.0.CO;2-1

SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (C) STRANGENESS: single

5 (i) MULTI-SENSE: NO

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

15 Ile Val Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys
1 5 10 15

20 (2) INFORMATION FOR SEQ ID NO: 29:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

35 (iii) HYPOTHETICAL: NO

40 (iv) ANTI-SENSE: NO

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

50 Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg Met Ala Trp Asp Met
1 5 10 15
Met Met Asn Trp
20

55 (2) INFORMATION FOR SEQ ID NO: 31:

60 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

65 (ii) MOLECULE TYPE: peptide

70 (iii) HYPOTHETICAL: NO

75 (iv) ANTI-SENSE: NO

80 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

85 Asn Thr Arg Gly Leu Val Ser Leu Phe Ser Pro Gly Ser Ala Gln Lys
1 5 10 15
Ile Gln Leu Val Asn
20

Claims

1. An antibody specifically binding to the C-terminal region of the HCV E1 protein (aa 227-383) or the N-terminal region of the HCV E2 protein (aa 384-450) which allows the *in situ* detection of HCV protein antigens.
2. The antibody of claim 1 wherein said antibody specifically binds to at least one of the following epitopes: -aa 307-326 of HCV E1 protein (SEQ ID 30)
-aa 395-415 of HCV E2 protein (SEQ ID 31).
3. The antibody of claims 1 and/or 2 wherein said antibody is a monoclonal antibody.
4. A monoclonal antibody secreted by the ECACC deposit having provisional accession number 98031215 or 98031214.
5. A functionally equivalent variant or fragment of an antibody according to claims 1 to 4.
6. A hybridoma cell line secreting a monoclonal antibody which specifically binds to the C-terminal region of the HCV E1 protein (aa 227-383) or the N-terminal hypervariable region of the HCV E2 protein (aa 384-450) and which allows the *in situ* detection of HCV protein antigens.
7. The hybridoma cell line of claim 5 wherein said hybridoma cell line is the ECACC deposit having provisional accession number 98031215 or 98031214.
8. A method for the *in situ* detection of HCV protein antigens comprising:
 - contacting a test sample which may contain HCV protein antigens with an antibody according to claims 1 to 4 or with a functionally equivalent variant or fragment of said antibody, to form an antibody-antigen complex, and
 - determining said antigen-antibody complex with an appropriate marker.
9. The method of claim 8 wherein said test sample comprises human cells or tissues.
10. The method of claim 9 wherein said human cells are peripheral blood cells.
11. The method of claim 9 wherein said human tissue is liver tissue.
12. An assay kit for the *in situ* detection of HCV protein antigens comprising:
 - an antibody according to claims 1 to 4, or, a functionally equivalent variant or fragment of said antibody, and
 - appropriate markers which allow to determine the complexes formed between HCV protein antigens in a test sample with said antibody or a functionally equivalent variant or fragment of said antibody.

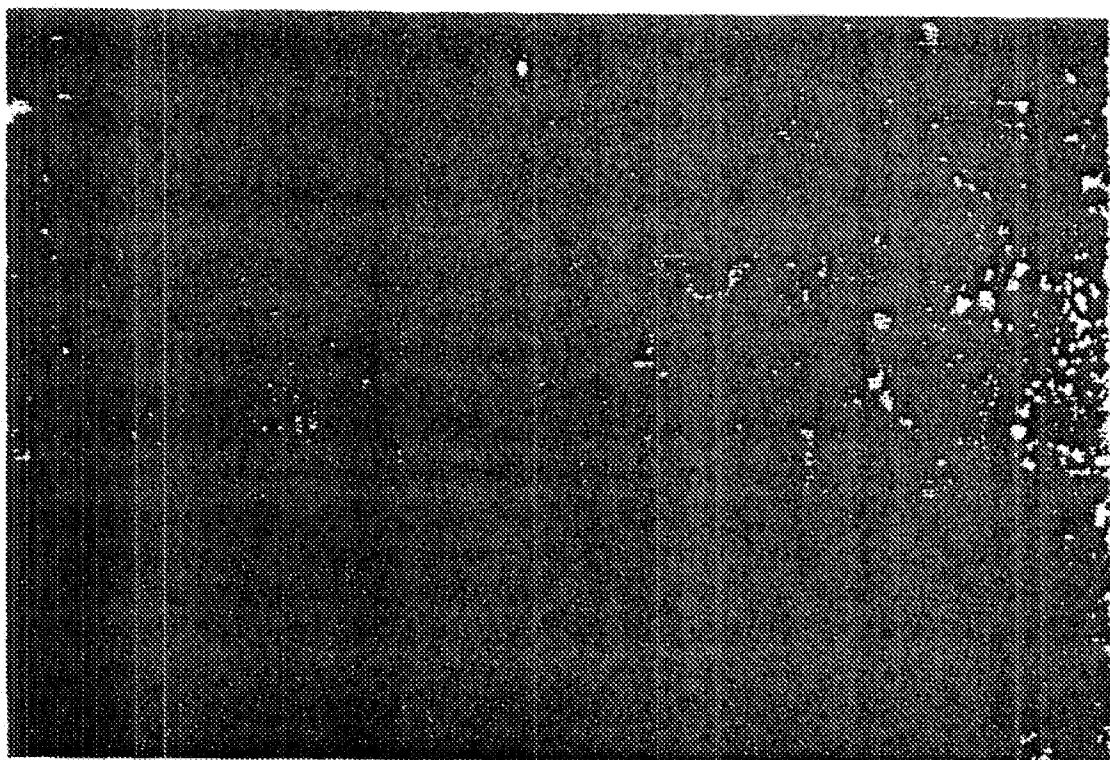


Figure 1

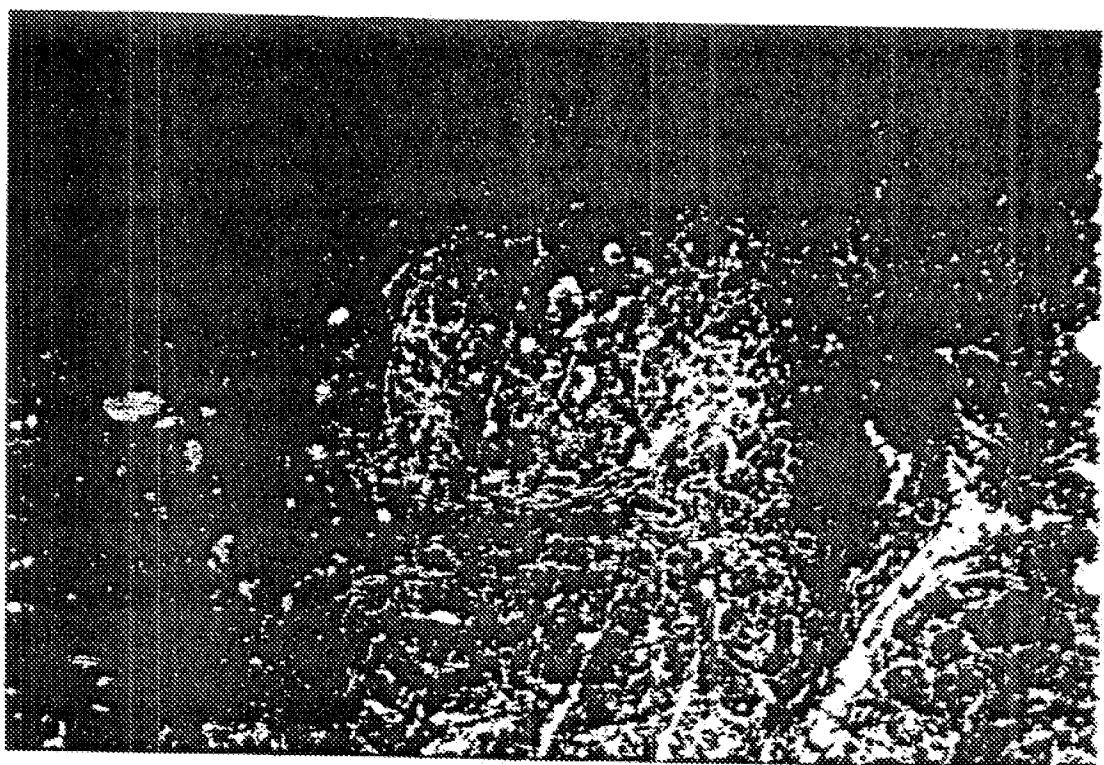


Figure 2

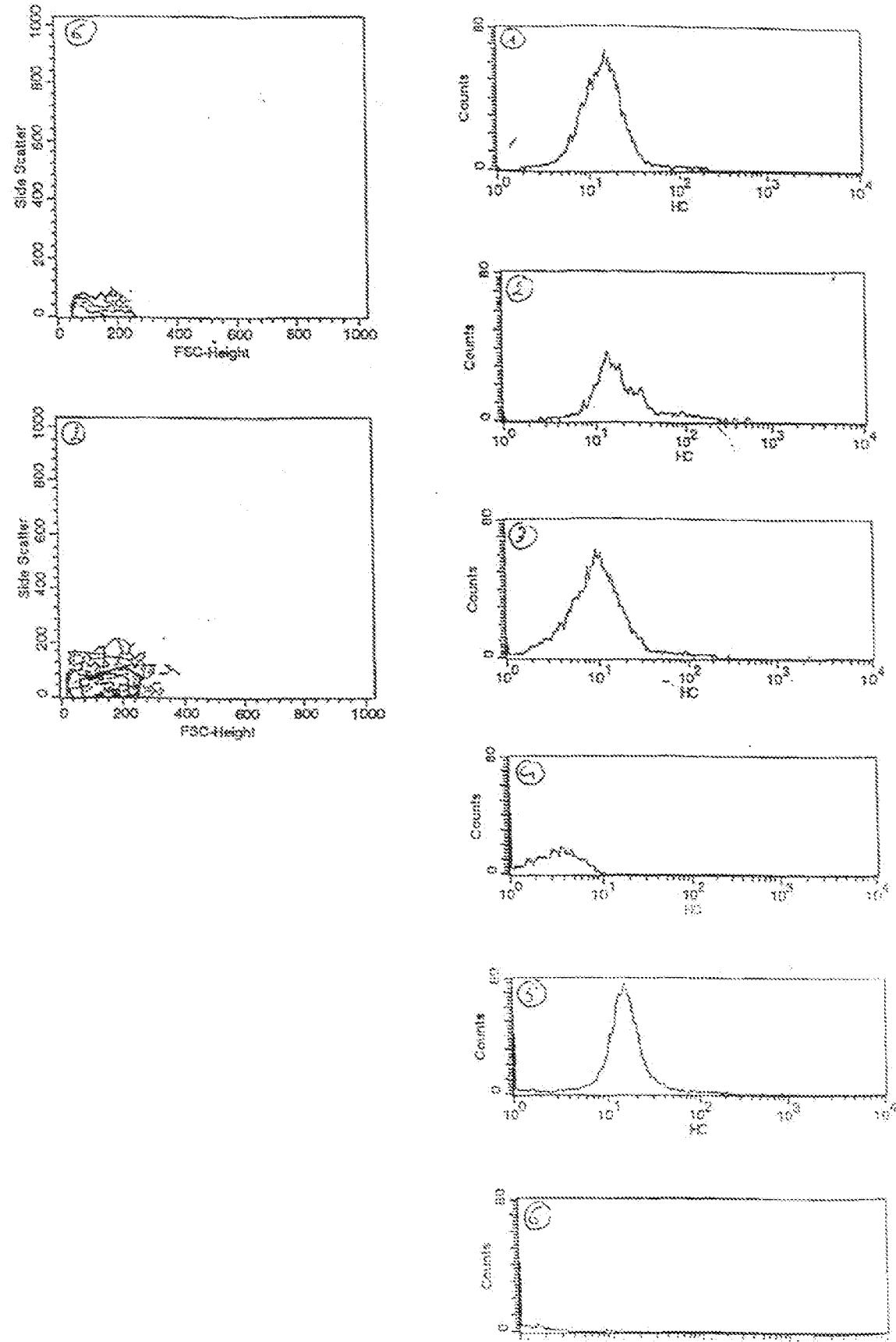


Figure 3

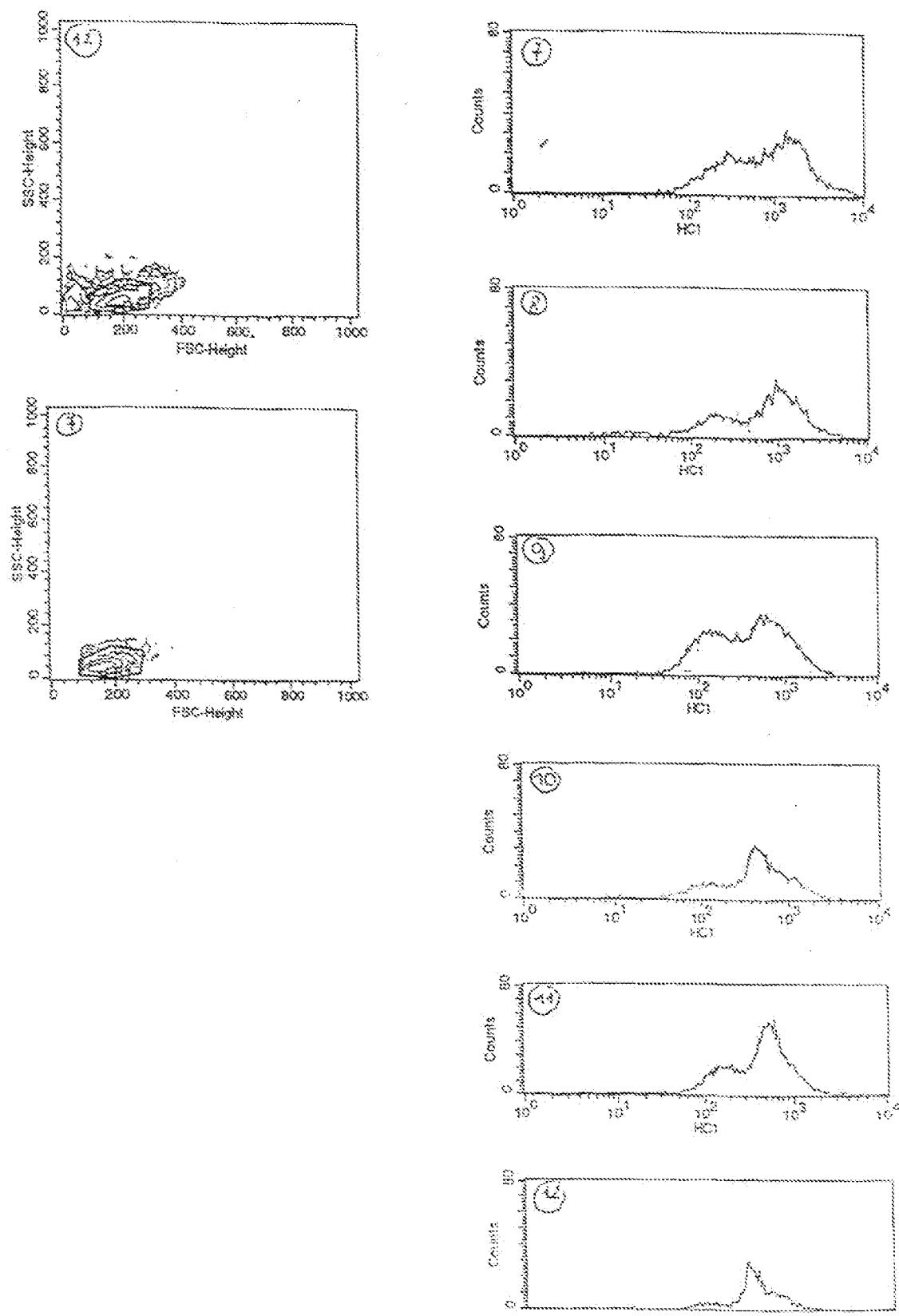


Figure 4



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 98 87 0058

DOCUMENTS CONSIDERED TO BE RELEVANT					
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC/US)		
X	WO 92 13892 A (ABBOTT LAB) 20 August 1992 * page 2, line 32 - page 3, line 4 * * page 8, line 21-31 * * page 12, line 35 - page 13, line 7 * * page 13; table 2 * *** WO 94 05311 A (DEAKIN RES LTD ;COMIS ALFIO (AU); FISCHER PETER (NO); TYLER MARGAR) 17 March 1994 * page 9, line 16-32 * * seq. ID 8 * page 30, line 31 - page 31, line 4 * * page 16, line 3-7 * * example 3 *	1-10	C07K16/10 C12N5/20 G01N33/576 //C07K14/08		
X	DE 42 09 215 A (BOEHRINGER MANNHEIM GMBH) 7 January 1993 * page 2, line 40-42 * * page 2, line 56 * * page 4, line 55 - page 5, line 1 * * page 29 seq. ID 12 * claims 1,11 *	1-10			
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Place of search	Date of conclusion of the search	Examiner			
THE HAGUE	20 November 1998	Covone, M			
CATEGORY OF CITED DOCUMENTS					
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background G : non-written disclosure P : intermediate document					
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European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 98 87 0060

DOCUMENTS CONSIDERED TO BE RELEVANT								
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim						
A	<p>MAERTENS G. ET AL.: "MAPPING OF HUMAN AND MURINE B CELL EPITOPES ON PURIFIED HEPATITIS C VIRUS E1 AND E2 PROTEINS" "AASLD ABSTRACTS" HEPATOLOGY, vol. 25, no. 4part2, October 1997, page 186A XP002084941</p> <p>A232 * the whole document *</p> <p>***</p>	1-8						
A	<p>HIRAMATSU N. ET AL.: "IMMUNOHISTOCHEMICAL DETECTION OF HEPATITIS C VIRUS-INFECTED HEPATOCYTES IN CHRONIC LIVER DISEASE WITH MONOCLONAL ANTIBODIES TO CORE, ENVELOPE AND NS3 REGIONS OF THE HEPATITIS C VIRUS GENOME" HEPATOLOGY, vol. 16, no. 2, 1992, pages 305-311, XP002084942</p> <p>* abstract *</p> <p>* page 308, left-hand column, line 27 - right-hand column, line 13 *</p> <p>***</p>	8-10						
A	<p>CHAN S-W. ET AL.: "HUMAN RECOMBINANT ANTIBODIES SPECIFIC FOR HEPATITIS C VIRUS CORE AND ENVELOPE E2 PEPTIDES FROM AN IMMUNE PHAGE DISPLAY LIBRARY" J.GENERAL VIROLOGY, vol. 77, 1996, pages 2531-2539, XP002084943</p> <p>* page 2532, left-hand column, line 32-35</p> <p>* page 2533; table 1 *</p> <p>* page 2537, right-hand column, line 18-27</p> <p>*****</p>	1-7						
<p>The present search report is based on the search for all claims</p> <table border="1"> <tr> <td>Place of search</td> <td>Date of completion of the search</td> <td>Examiner</td> </tr> <tr> <td>THE HAGUE</td> <td>20 November 1998</td> <td>Covone, M</td> </tr> </table> <p>EEG/P36A 1523 02 82 P 04201</p> <p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background C : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date G : document cited in the application L : document cited for other reasons B : member of the same patent family, corresponding document</p>			Place of search	Date of completion of the search	Examiner	THE HAGUE	20 November 1998	Covone, M
Place of search	Date of completion of the search	Examiner						
THE HAGUE	20 November 1998	Covone, M						

**CLAIMS INCURRING FEES**

The present European patent application comprised at the time of filing more than ten claims.

Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s);

No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims: